injections of LPS (and after the initial cytokine release) also prevented organ injury and death. In contrast, intervention with anti-TNF antibody did not provide significant protection. These results support the concept that blocking CD14 in human septic shock may limit hypotension, disseminated intravascular coagulation and organ injury resulting from cumulative exposures to LPS.

Immune reconstitution in HIV disease

S58 Antiviral therapy of HIV infection

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In developed countries, potent combination therapy has led to important reductions in morbidity, mortality and hospitalization. However, at the same time, as always, we face new challenges. To what extent these effects will be sustained is not known. Resources and outpatient facilities are being 'stretched'. Poor compliance with complicated drug treatments requires a simplification of regimens. There is evidence of low-level replication continuing in the face of an apparent 'suppression' of replication, and together with sanctuary sites of virus division, the emergence of resistance in the longer term seems inevitable. Resistance may cross drug classes, reducing therapeutic options for change. Immune reconstitution is largely partial, heightening interest in supplementary immune-based therapies. Resistance testing is 'leaking' into clinical practice before we really know how useful it is, and when to start therapy, when to change, what to use and how best to sequence drugs are vital questions that we need to address.

S59 Maintenance and prophylactic treatment of opportunistic infections during HAART

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HAART has profoundly affected the incidence of common AIDS-related opportunistic infections (OIs) in countries where this therapy is widely available. In the USA and western Europe, the incidence of OIs such as *Pneumocystis carinii* pneumonia (PCP), disseminated *Mycobacterium avium* complex infection (MAC) and cytomegalovirus end-organ disease (CMV) is now 20% of that in 1994. However, the incidence does not appear to have decreased further since 1997. There have been several recent reports of individual AIDS patients with MAC and CMV on maintenance therapy for these OIs who, after their CD4 count rose to 100 cells/(L on HAART, have discontinued maintenance therapy without OI relapse after up to 1 year of follow-up. There are also reports of small series of patients who were receiving PCP or MAC prophylaxis for CD4 counts <200 or 50 and who, after their CD4 count rose to 200 or 100 cells/(L on HAART, have discontinued prophylaxis without developing these OIs. However, it is not possible to accurately predict which patients with prior low CD4 counts who have responded to HAART can safely discontinue OI maintenance or prophylaxis treatment. Observational studies are in progress to better define markers of OI protection. These include pathogen-specific immune function and T-cell phenotype assays in addition to standard CD4 and I-UV RNA measurements. Pending results of these trials, continued maintenance and prophylaxis should be based on nadir CD4 counts unless the risk of anti-OI drug adverse effects is substantial.

Thinking like bacteria—the modern approach to overcome resistance in Gram-negative bacteria

S61 Evolution of resistance mechanisms—intelligent bacterial solutions to combat antibiotic attack

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It is generally assumed that a pool of resistance genes evolved in antibiotic-producing microorganisms before antibiotics were used for treatment of infectious diseases. These genes code for antibiotic-inactivating enzymes, like beta-lactamases, as well as target-modifying enzymes like rRNA methylases. Moreover, efflux pumps reducing the intracellular drug concentration have evolved with narrow or broad substrate specificities. The spread of the corresponding resistance genes among different bacteria seems to have been primarily influenced by the local selective pressure within the natural environment. However, during the last decades conditions of selection have changed rapidly due to the intensive use of antibiotics and the development of new drugs. But within a short period of time, bacteria have developed effective defense mechanisms against every new drug introduced. Besides the transfer of preformed resistance determinants, point mutations have played a major role in this short-term resistance development. These point mutations have affected not only genes coding for already existing inactivating enzymes (*aacA*, *bla*) or regulatory elements controlling efflux pumps (*norA*, *mex*), but also genes coding for targets of antibiotic action. These include genes like *rpoB*, coding for the target of rifampin, or genes *gyrA* and *parC*, coding for the A subunits of type II topoisomerases, DNA gyrase and topoisomerase IV, respectively. These are the targets inhibited by the synthetic quinolones. Due to the high antibacterial activity of new fluorinated quinolones against *E. coli*, a combination of mutations is required for clinically relevant levels of resistance. Observations with laboratory strains suggest that the accumulation of resistance mutations simultaneously reduces the viability. Thus, additional mutations, not necessarily affecting resistance, are assumed to restore fitness, as has been observed in fluoroquinolone-resistant clinical isolates.

S62 Quorum sensing: the language of Gram-negative bacteria

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The discovery of *N*-acyl-L-homoserine lactone molecules as the signaling mechanism in *Photobacterium (Vibrio) fischeri* to sense and respond to their own density in the light organs of certain fish in order to bring about the quorum response of bioluminescence has led to the elucidation of similar mechanisms in an ever-increasing number of Gram-negative organisms—to the extent that quorum sensing appears to be a universal language of Gram-negative bacteria. In at least one human pathogen, *Pseudomonas aeruginosa*, quorum sensing appears to control the expression of numerous virulence factors such as elastase, exotoxin A, pyocyanin, alkaline protease, cyanide and hemolysin. Interestingly, there even appears to be the capability of interspecies communication, with *Burkholderia cepacia* being able to sense and respond to the density of *Pseudomonas aeruginosa* in the lungs of cystic fibrotic patients. Using a model based on the luminescence operon of *Photobacterium fischeri*, several potential mechanisms to disrupt quorum sensing, and, hence, pathogenicity, may be possible. Currently, the most attractive of these is to use compounds resembling the *N*-acyl-L-homoserine lactone

molecules, which then act in competition for the activation sites of the operons or regulons under quorum sensing control. Control of pathogenicity in this way may offer a unique form of antimicrobial therapy without the endotoxemia associated with Gram-negative cell lysis.

S63 A new approach for controlling bacterial populations (sonic communication with bacteria)

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Introduction and objectives: We previously reported that bacteria communicate with each other by using growth-regulating sonic/ultrasonic signals (Matsuhashi et al, J Gen Appl Microbiol 1996; 42: 315–323). *Bacillus subtilis* cells emit sounds at frequencies of about 8.5 kHz and its overtones, and the growth of *B. carboniphilus* cells is promoted by sounds/ultrasounds from a speaker at similar frequencies under non-permissive stress conditions (Matsuhashi et al, J Gen Appl Microbiol 1998; 44: 49–55). Graphite, charcoal and other materials convert external energy, such as pulses of infrared light, into sonic signals (Matsuhashi et al, J Gen Appl Microbiol 1997; 43: 225–230). The response of bacteria to sounds may be utilized in antibiotic therapy.

Results: (1) Soft solid matter, such as solidified agar in growth media, can significantly promote or inhibit the growth of bacteria and yeast under stress conditions and the hatching of fish eggs. The effect is probably due to photo-acoustic emission resulting from the conversion of external electromagnetic energy and can be diminished by shielding the agar and organisms with aluminum foil. *E. coli* and yeasts also responded to signals from the agar. This mechanism probably explains why many bacteria and mycoplasmas grow well on soft solid surfaces such as animal tissue. (2) In contrast to *B. carboniphilus*, soft low-frequency sounds from a speaker, at frequencies between 10 and 800 Hz, markedly inhibit the growth of *E. coli*. This *E. coli* behavior, which may be common in related Gram-negative bacilli, suggests that low-frequency sounds may help to overcome infections by Gram-negative bacteria.

S64 Quorum sensing—a new target for antibiotic therapy

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There are two broad strategies for controlling bacterial infection: either (1) kill the organism or (2) attenuate virulence such that the infecting oragnism fails to adapt to the host environment and can be cleared by the innate host defenses. The latter approach has, until recently, lacked specific targets for rational drug design. However, the discovery that bacterial cells communicate with each other using small diffusible signaling molecules to regulate virulence in concert with cell population density (termed quorum sensing) now offers such a novel target.

Many Gram-negative bacteria employ *N*-acylhomoserine lactones (AHLs) as quorum sensing signaling molecules which not only control gene expression but also possess potent pharmacologic activities such that they may function as virulence determinants *per se*. Understanding the molecular mechanisms by which AHLs are produced (usually via a member of the Lux family of AHL synthases) and by which AHLs activate target gene expression (via members of the LuxR family of transcriptional regulators) is central to the design of small-molecule antagonists (Quorum Sensing Blockers, QSBs) capable of attenuating virulence through the blockade of either signal generation or signal transduction.

Toxicity of antimicrobial agents: relevance of preclinical data for the clinician

S67 Immunologic reactions to antimicrobial agents

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This presentation reviews the incidence, clinical manifestations, differential diagnosis, risk factors and pathogenesis of allergic reactions of two important classes of antimicrobials; beta-lactams and sulfonamides. The diagnostic work-up of a patient with a history of an allergic reaction will be discussed, as well as the possibility of the safe administration of the drug in the face of an allergy using immunotherapy. Emerging concepts of beta-lactam side-chain allergy, the role of cellular immune mechanisms and the clinical importance of cross-reactivity of allergic reactions to different classes of beta-lactams will be emphasized.

S68 Toxic effects of antimicrobial agents on the musculoskeletal system

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Several groups of antimicrobial agents have the potential to have adverse effects on the musculoskeletal system. At the beginning of the 1960s, the effects of tetracycline on the developing skeleton were investigated in premature infants by multiple X-ray examinations. Based on these results, tetracylines were considered to be contra-indicated in infants and children. Quinolones are another group of frequently used drugs that have toxic effects on connective tissue structures. Due to their cartilage toxicity—as shown in immature dogs and other animals—they are contraindicated in children and adolescents. Human experience with pefloxacin in children showed an unacceptably high incidence of joint toxicity (10%). However, with nalidixic acid, norfloxacin and ciprofloxacin, favorable clinical results in juvenile patients have been published. Also, quinolones have the potential to induce tendon disorders (tendinitis, Achilles tendon rupture) which have occurred mainly in elderly patients. Mechanistic studies from our group indicate that the magnesium-chelating properties of the quinolones might be responsible for these toxic effects. Less often used antimicrobial drugs which might affect the musculoskeletal system include, for example, pyrazinamide, rifabutin, and quinupristin–dalfopristin.

Infectious disease problems in Central and Eastern Europe

S71 Typical problems of infectious diseases in Central Europe

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Central and eastern European countries face the same types of